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PATENT

Attorney Reference Number 4239-61854
Application Number

REMARKS

This Preliminary Amendment is submitted to recite the priority claim from corresponding International Application No. PCT/US00/19039 filed July 12, 2000, which claims the benefit of U.S. Provisional Application 60/143,560 filed July 13, 1999 and U.S. Provisional Application 60/157,471 filed October 1, 1999. No new matter is added.

This replaces the priority claim present in the PCT application.

This Preliminary Amendment also implements changes to the specification made under an Article 34 Amendment filed in the PCT application. These amendments were made to insert sequence identifiers.

CONCLUSION

If any minor matters remain to be discussed prior to examination, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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**Marked-up Version of Amended Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)**

In the specification:

In the specification, on page 1 after the title, please add the following paragraph:

PRIORITY CLAIM

This is a § 371 U.S. national stage of PCT/US00/19039 filed July 12, 2000, which was published in English under PCT Article 21(2), which in turn claims the benefit of U.S. Provisional Application 60/143,560 filed July 13, 1999 and U.S. Provisional Application 60/157,471 filed October 1, 1999.

Please amend pages 10, 52 and 53 as shown in the attached pages.

FIGURE 13. TARP exists in the nuclei of breast cancer cells (A) Western blot of nuclear extracts derived from LNCaP, MCF7, BT-474, SK-BR-3 and Hs57BsT cells. 40 µg of each nuclear extract were run on a 16.5% Tris-Tricene gel and probed with an antibody against TARP (upper panel) or TCRγ (bottom panel). As a positive control, 1 µg of His-tagged TARP (His-TARP) and 100 ng of His-tagged TCRγ (His-TCRγ) were run on the gels. Size markers in kDa are indicated on the left.

FIGURE 14. Potential functional domains of TARP. (A) TARP (SEQ ID NO:14) contains a potential leucine zipper motif and phosphorylation sites. A potential leucine zipper motif is indicated with boxed leucines followed by a basic region that is underlined. cAMP- and cGMP-dependent protein kinase phosphorylation sites (amino acids 46-49 and 55-58) and protein kinase C phosphorylation sites (amino acids 19-21 and 20-22) are outlined. (B) Protein sequence comparison of TARP with Tup1. Amino acids sequences for TARP (42-57) (SEQ ID NO:16), *Dictyostelium discoideum* Tup1 (dTup1, 521-536) (SEQ ID NO: 17) and *Saccharomyces cerevisiae* Tup1 (yTup1, 626-660) (SEQ ID NO:18) are shown. Conserved residues are boxed.

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DETAILED DESCRIPTION OF THE INVENTION

I. INTRODUCTION

Surprisingly, it has been discovered that prostate cells of epithelial origin, and cells of many breast [cancer s] cancers, express mRNA of the T-cell receptor gamma chain ("TCRγ"). The major TCRγ transcript in prostate has a different size than that expressed in T lymphocytes. The findings that prostate epithelial cells and many breast cancers express a high level of a transcript from a gene thought to be expressed exclusively in T lymphocytes is highly unexpected.

Because the TCRγ reading frame contains a good Kozak sequence (Kozak, M. *Cell* 44:283-92 (1986)), we initially hypothesized that a truncated TCRγ protein was encoded. Thus, it was an additional surprise to find that the TCRγ locus expressed in epithelial prostate cancer cells and breast cancer cells encodes a 7 kDa nuclear protein. Because the protein is encoded from a reading frame different from TCRγ, we have named it "TARP," for TCRγ Alternate Reading frame Protein. Besides being translated from an alternate reading frame of a transcript originating within an intron of

the TCR γ locus, TARP has two other unusual features. First, it is surprising to find such a small peptide in the cell because most are usually secreted. Second, TARP lacks a good Kozak sequence

any non-lymphoid-derived cell type, express high level of a transcript from a gene that was thought to be exclusively expressed by cells from the lymphoid lineage, was a highly unexpected discovery.

EXAMPLE 2. DISCOVERY OF THE TCR γ ALTERNATE READING FRAME PROTEIN

5 The previous Example demonstrated the unexpected discovery of TCR γ transcript in prostate and prostate cancer cells, the in vitro translation of the transcript, and the initial hypothesis that the transcript resulted in the presence of a truncated form of TCR γ chain in these cells. This Example sets forth the further unexpected discovery that the transcript in fact results in a previously unknown protein, now designated "TARP," expressed from an alternate reading frame. Even more
10 unexpectedly, the studies reported below show that TARP is a nuclear protein, and is present in many breast cancer cells.

MATERIALS AND METHODS

Primers. (SEQ ID NOs: 19-31) TCR γ -upATGmut#1 (5'-TTACAGATAAACAA
15 CTTGATACAGATGTTTCCCCCAAGCCC-3'); TCR γ -upATGmut#2 (5'-GGGCTTGGGGGAAA
CATCTGTATCAAGTTGTTTATCTGTAA-3'); TCR γ -upATGmut#3 (5'-GATAAACAACTTGA
TGCAGATATTTCCCCCAAGCCC-3'); TCR γ -upATGmut#4 (5'-GGGCTTGGGGGAAATATCTG
CATCAAGTTGTTTATC-3'); TCR γ -upATGmut#5 (5'-GATAAACAACTTGATACAGATA'TTT
CCCCCAAGCCC-3'); TCR γ -upATGmut#6 (5'-GGGCTTGGGGGAAATATCTGTATCAAGTTG
20 TTTATC-3'); TCR γ -downATGmut#1 (5'-CCCAGGAGGGGAACACCATAAAGACTAACGAC
ACATAC-3'); TCR γ -downATGmut#2 (5'-GTATGTGTTCGTTAGTCTTTATGGTGTTCCTCC
TGGG-3'); TCR5.1 (5'-GATAAACAACTTGATGCAGATGTTTCC-3'); TCR3.1 (5'-TTATGATT
CTCTCCATTGCAGCAG-3'); TCR γ 1.2R (5'-AAGCTTTGTTCCGGGACCAAATAC); B-Actin
Forward (5'-ATCTGGCACCACCTTCTACAATGAGCTGCG-3'); B-Actin Reverse (5'-CTTC
25 ATAC'CTCTGCTTGCTGATCCACATCTGC-3'). Primers were synthesized by Sigma-Genosys
(The Woodlands, TX) and Lofstrand Labs Limited (Gaithersburg, MD).

Constructs. The *TARP* transcript cloned into pBluescript II SK(+) (Stratagene, La Jolla, CA) was described previously (Essand, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 96:9287-9292 (1999)). This plasmid is referred to as pBSSK-TCR γ in this

TARP may only be functioning as a nuclear localization signal, but the fact that TARP is a nuclear protein strengthens the hypothesis that TARP may bind DNA. Functional studies are needed before any definitive conclusions can be made.

To determine if TARP shares homology with any known proteins, we performed a protein BLAST search against GenBank. This search indicated that the amino acid sequence of TARP shares some homology to *Dictyostelium discoideum* Tup1 (GenBank accession no. AAC29438) and *Saccharomyces cerevisiae* Tup1 (Williams, F. E. *et al.*, *Mol. Cell. Biol.* 10:6500-6511 (1990)) (Figure 7C). Yeast Tup1 is normally found in a complex with Cyc8(Ssn6) and is required for transcriptional repression of genes that are regulated by glucose, oxygen and DNA damage (Tzamarias, D. *et al.*, *Genes Dev.* 9:821-831 (1995)). Neither Cyc8(Ssn6) nor Tup1 binds DNA, but each acts as a part of a corepressor complex through interactions with specific DNA-binding proteins such as $\alpha 2$, Mig1, Rox1 and a1 (Tzamarias, D. *et al.*, *Genes Dev.* 9:821-831 (1995)). The C'-terminal half of Tup1 contains six repeats of a 43-amino acid sequence rich in aspartate and tryptophan, known as WD-40 or β -transducin repeats (Williams, F.E. *et al.*, *Mol. Cell. Biol.* 10:6500-6511 (1990); Fong, H.K. *et al.*, *Proc. Natl. Acad. Sci. USA* 83:2162-2166 (1986)). WD-40 repeats have been identified in many proteins and play a role in protein-protein interactions. Importantly, Tup1 has been shown to interact with $\alpha 2$ through two of its WD-40 repeats (Komachi, K. *et al.*, *Genes Dev.* 8:2857-2867 (1994)). It is interesting to note that TARP shares homology with the fifth WD-40 repeat of Tup1 (Figure 7C). Because TARP is a nuclear protein, its homology with Tup1 suggests that TARP may be a member of a functional nuclear protein complex involved in transcriptional regulation. Therefore, it is necessary to identify TARP-interacting proteins in order to determine its function.

The TARP antibody recognizes a doublet in prostate and breast nuclear extracts (Figure 6A). The faster 7 kDa band comigrates with the His-TARP recombinant protein, while the weaker band runs at a larger molecular weight. One possible explanation for the 9 kDa band is post-translational modifications. To determine if TARP contains any known post-translational modification sites, we analyze the TARP amino acid sequence using the PROSITE program of the Swiss Institute of Bioinformatics ExPASy proteomics server (<http://www.expasy.ch>) (Appel, R.D. *et al.*, *Trends Biochem. Sci.* 19:248-260 (1994); Hofmann, K *et al.*, *Nucleic Acids Res.* 27:215-219 (1999)). As shown in Figure 7A, many potential phosphorylation sites were found including cAMP-

and cGMP-dependent protein kinase phosphorylation sites (RRAT (SEQ ID NO:32) and RRGTT (SEQ ID NO:33)) and